

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

RUNGE et al.

U.S. Application Serial No. : 10/058,022

Filed : 01/29/2002

For : „A process for producing dry powders of one or more carotenoids“

DECLARATION

I, Jesper Feldthusen Jensen, Dr. rer. nat., a citizen of Denmark and residing at Grauelstrasse 21, D-55129 Mainz, Federal Republic of Germany declare as follows:

I am a fully trained chemist, having studied chemistry at The Technical University of Denmark, Lyngby, Denmark, from 1989 to 1994, Master in Chemical Engineering 1994; followed by a Ph.D. study at The University of Mainz, Federal Republic of Germany from 1994 to 1998.

I obtained my doctor's degree from The University of Mainz in 1998. I joined BASF Aktiengesellschaft, D-67056 Ludwigshafen, Federal Republic of Germany, in 1999 (after a 1 year Post-Doc study at The University of Australia) since when I have been working mainly in the field of polymer science (from 1999-2002) and formulation of carotenoids (animal nutrition) (from 2002-).

I work in the fine chemical formulation group of BASF's (animal nutrition business unit) and am therefore familiar with the field to which the said application relates.

I studied the Office Actions mailed August 11th, 2003, and April 20th, 2004 wherein the Examiner has rejected our claims 1 to 14 as being unpatentable as obvious over Jensen et al. (WO 91/06292) in view of Horn et al (US 4,522,743) or Dobler et al. (WO 96/01570) respectively or in view of both, according to 25 USC 103(a).

Our invention aims at the increase of stability. To demonstrate this we have conducted experiments which further corroborate that the use of at least one soybean protein in combination with lactose is essential in the preparation of dry powders comprising one or more carotenoids and that the combined use of the soy bean protein(s) and lactose conveys properties to the dry powder which are distinctly different from the properties of dry powders which are obtained when another protective colloid and/or another sugar are employed.

More particularly, we have prepared astaxanthin dry powders in the manner described for Example 1, page 11, indicated lines 17 to 41, of the application:

- A first sample (A) representing our invention was prepared using soybean protein instead of the partially degraded soybean protein employed in Example 1.
- A second sample (B) was prepared for comparison using soybean protein instead of the partially degraded soybean protein employed in Example 1, and using glucose instead of the lactose employed in Example 1.
- A third sample (C) was prepared for comparison using casein instead of the partially degraded soybean protein employed in Example 1.
- A fourth sample (D) was prepared for comparison using gelatine instead of the partially degraded soybean protein employed in Example 1.

After determining the astaxanthin content of the dry powders (A) to (D), the samples were stored at 60°C for a period of 40 days. After the storage period the astaxanthin content of the dry powders (A) to (D) was measured again. The respective data are compiled in the following table:

Sample	Protective Colloid / Sugar	Carotenoid Content before Storage (%)	Carotenoid Content after Storage (%)	Carotenoid Loss upon Storage (%)
(A)	Soybean protein / lactose	13.7	11.4	16.5
(B)	Soybean protein / glucose	13.2	9.2	30.2
(C)	Casein / lactose	13.1	8.8	32.5
(D)	Gelatine / lactose	11.3	5.9	48.3

The data show that the nature of the protective colloid and of the sugar, which are employed in combination with each other has a distinct and unexpected impact on the storage stability of the carotenoid dry powder. When the soybean protein / lactose combination in accordance with applicants' invention as illustrated by sample (A) was used, the carotenoid content of the dry powder was reduced upon storage by 16.5%. However, when the lactose was replaced by glucose as illustrated by sample (B), the carotenoid loss upon storage amounted to 30.2% which is almost twice the loss suffered by sample (A). Similarly, when lactose was used as the sugar but the soybean protein was replaced by another protective colloid as illustrated by samples (C) and (D), the carotenoid loss upon storage increased to 32.5% and 48.3%, respectively.

Example A:

Preparation of an astaxanthin powder product based on the combination of soy protein and lactose.

48 g of crystalline astaxanthin, 1,6 g of ascorbyl palmitate and 20 g of alpha-tocopherol were suspended in 350 g of an azeotropic isopropanol/water mixture at room temperature in a heatable container. The active substance suspension was heated to 90 °C and mixed at a flow rate of 2.1 kg/h continuously with further isopropanol/water azeotrope at a temperature of 224 °C with a flow rate of 2.7 kg/h, the astaxanthin dissolving at a mixing temperature of 165 °C, which was set up, under a pressure of 55 bar. This active substance solution was immediately mixed with an aqueous phase consisting of a solution of 67.68 g Soy protein, 198.04 g lactose, and 5.4 g of a preservative mixture in 10547.5 g of distilled water, in which the pH was adjusted to 9.5 with 1 M NaOH, at a flow rate of 60 kg/h.

The active substance suspension produced on mixing had an $E_{1/1}^{1\%}$ value of 127.

The active substance suspension was then concentrated in a thin film evaporator to a concentration of about 5.24 % of active substance content ($E_{1/1}$ value of 126) and spray dried. The dry powder had an astaxanthin content of 13.7 %. The dry powder re-dispersed in water had an $E_{1/1}$ value of 121.

¹⁾ The $E_{1/1}$ value defines in this connection the specific extinction of a 1 % strength aqueous dispersion of a 10 % by weight dry powder in a 1 cm cuvette at the absorption maximum.

Comparative example B:

Preparation of an astaxanthin powder product based on the combination of soy protein and glucose DE 40.

48 g of crystalline astaxanthin, 1,6 g of ascorbyl palmitate and 20 g of alpha-tocopherol were suspended in 350 g of an azeotropic isopropanol/water mixture at room temperature in a heatable container. The active substance suspension was heated to 90 °C and mixed at a flow rate of 2.1 kg/h continuously with further isopropanol/water azeotrope at a temperature of 225 °C with a flow rate of 2.7 kg/h, the astaxanthin dissolving at a mixing temperature of

166 °C, which was set up, under a pressure of 55 bar. This active substance solution was immediately mixed with an aqueous phase consisting of a solution of 67.68 g Soy protein, 198.00 g glucose DE 40, and 5.4 g of a preservative mixture in 10547.5 g of distilled water, in which the pH was adjusted to 9.5 with 1 M NaOH, at a flow rate of 60 kg/h.

The active substance particles produced on mixing had a particle size of 126 nm in the isopropanol/water mixture, with an $E1/1^{11}$ value of 125.

The active substance suspension was then concentrated in a thin film evaporator to a concentration of about 3.53 % of active substance content ($E1/1$ value of 108) and spray dried. The dry powder had an astaxanthin content of 13.2 %. The dry powder re-dispersed in water had a particle size of 351 nm and an $E1/1$ value of 99.

Comparative example C:

Preparation of an astaxanthin powder product based on the combination of Na-caseinate and lactose.

48 g of crystalline astaxanthin and 20 g of alpha-tocopherol were suspended in 351.6 g of an azeotropic isopropanol/water mixture at room temperature in a heatable container. The active substance suspension was then heated to 90 °C and mixed at a flow rate of 2.1 kg/h continuously with further isopropanol/water azeotrope at a temperature of 225 °C with a flow rate of 2.69 kg/h, the astaxanthin dissolving at a mixing temperature of 166 °C, which was set up, under a pressure of 55 bar. This active substance solution was immediately mixed with an aqueous phase consisting of a solution of 74.98 g Na-caseinate, 220.99 g lactose, and 5.99 g of a preservative mixture in 11686.6 g of distilled water, in which the pH was adjusted to 9.5 with 1 M NaOH, at a flow rate of 60 kg/h.

The active substance particles produced on mixing had a particle size of 143 nm in the isopropanol/water mixture, with an $E1/1^{11}$ value of 128.

The active substance suspension was then concentrated in a thin film evaporator to a concentration of about 1.3 % of active substance content ($E1/1$ value of 126) and spray dried. The dry powder had an astaxanthin content of 13.1 %. The dry powder re-dispersed in water had a particle size of 153 nm and an $E1/1$ value of 127.

Comparative example D:

Preparation of an astaxanthin powder product based on the combination of gelatin and lactose.

24 g of crystalline astaxanthin, 2.0 g of ascorbyl palmitate, and 10 g of alpha-tocopherol were suspended in 172.7 g of an azeotropic isopropanol/water mixture at room temperature in a heatable container. The active substance suspension was then heated to 89 °C and mixed at a flow rate of 2.1 kg/h continuously with further isopropanol/water azeotrope at a temperature of 230 °C with a flow rate of 2.757 kg/h, the astaxanthin dissolving at a mixing temperature of 168 °C, which was set up, under a pressure of 55 bar. This active substance solution was

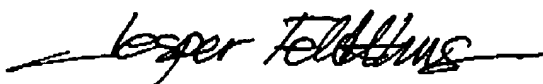
immediately mixed with an aqueous phase consisting of a solution of 11.68 g gelatin B 100 bloom, 53.4 g Gelita Sol PA (hydrolysate of gelatin B), 96.22 g lactose, and 2.7 g of a preservative mixture in 5882.55 g of distilled water, in which the pH was adjusted to 9.5 with 1 M NaOH, at a flow rate of 60.958 kg/h.

The active substance particles produced on mixing had a particle size of 104 nm in the isopropanol/water mixture, with an E1/1¹⁾ value of 124.

The active substance suspension was then concentrated in a thin film evaporator to a concentration of about 3.8 % of active substance content (E1/1 value of 128) and spray dried. The dry powder had an astaxanthin content of 11.3 %. The dry powder re-dispersed in water had a particle size of 241 nm and an E1/1 value of 125.

I further declare that all statements made herein of my own knowledge are true and that statements made on information or belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at D-67056 Ludwigshafen, Germany, October 28th, 2005



Signature of Declarant